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CITATION:

Itani, Gyo. Two types of symbioses between grapsid crabs and a host thalassinidean shrimp. PUBLICATIONS OF THE SETO MARINE BIOLOGICAL LABORATORY 2001, 39(2-3): 129-137

ISSUE DATE:

2001-03-31

URL:

<http://hdl.handle.net/2433/176297>

RIGHT:

## Two types of symbioses between grapsid crabs and a host thalassinidean shrimp

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**Abstract** Two species of grapsid crabs, both of the genus *Acmaeopleura*, were collected from burrows of a thalassinidean shrimp, *Upogebia major*, in a tidal flat in Japan. One was *Acmaeopleura toriumii*, co-habiting in the burrows, and the other was an undescribed species, *Acmaeopleura* sp., clinging to the abdomen of *U. major*. Several specimens of *A. toriumii* were also collected from egg masses of *U. major*. The field experiment and samplings suggested that *A. toriumii* is not a burrowing species and depends on *Upogebia* burrows. Behavioral observations were also made on crabs of the two species maintained in aquaria together with the shrimp. *Acmaeopleura toriumii* never clung to *U. major* and seemed to be an omnivorous feeder. In contrast, *Acmaeopleura* sp. always clung, and almost specifically to the ventral side of the first and second abdominal segments of the host; moreover this crab sometimes migrated to another host individual. A few days after *Acmaeopleura* sp. was observed clinging to a shrimp, wounds were observed on the abdomen of the host. This ectosymbiotic crab probably feeds on host tissue. In the field, about 13% of the collected *U. major* had abdominal wounds attributable to *Acmaeopleura* sp. Symbiotic relationship between *Acmaeopleura* sp. and *U. major* will be the first case reported of a crab living ectoparasitically on another decapod.

**Key words:** *Acmaeopleura*, Brachyura, *Upogebia*, Thalassinidea, symbiosis, burrow commensal, ectoparasite

### Introduction

The infraorder Thalassinidea comprises decapod crustaceans that are adapted to a burrowing life-style (Poore, 1994). Among thalassinideans, members of the family Upogebiidae are common burrowers in sand and mud in temperate and tropical marine environments around the world. They construct U- or Y-shaped burrows and create water currents with their rhythmically stroking pleopods. Upogebiids feed mainly on suspended matter strained out of the water current by the setal basket formed by the first and second pereopods, but are equipped for resuspension feeding and deposit feeding as well (Dworschak, 1987; Griffis and Suchanek, 1991; Nickell and Atkinson, 1995; Coelho *et al.*, 2000).

Although upogebiid shrimps might reduce the abundance of macro-infauna in soft sediment community (Brenchley, 1981; Posey *et al.*, 1991), upogebiid burrows themselves are inhabited by a variety of organisms, such as bivalves, annelids, copepods, shrimps, crabs, and gobiid fishes (Norman, 1891; MacGinitie, 1930, 1935; Grossman, 1979; Rios and Carvacho, 1983). Upogebiid shrimps also serve as hosts for ectosymbiotic animals, such as bivalves and bopyrid isopods (MacGinitie, 1935; Kato and Itani, 1995, 2000).

Brachyuran crabs contain many symbiotic species (Dales, 1957; Sakai, 1976; Weinbauer *et al.*, 1982; Ross, 1983; Štević *et al.*, 1988; Vandenspiegel *et al.*, 1992). However, crabs known to be symbiotic with other crustaceans have been confined to burrow co-habitants. Some crabs of the family Pinnotheridae live in the burrows of upogebiid and other thalassinidean shrimps in the East Pacific and the West Atlantic (MacGinitie, 1930, 1935; Manning and Felder, 1989).

Grapsid crabs of the genus *Acmaeopleura* [Subfamily Varuninae] are known only from the Indo-West Pacific region and association of these crabs with upogebiid shrimps has been suggested (Ghani and Tirmizi, 1991; Davie, 1992; Sakai, 2000). Davie (1992) reported that *A. toriumii* occurred in mud tunnels of either *Upogebia major* or the echiuroid *Ochetostoma erythrogrammon*.

from Hong Kong. But ascertaining whether the crabs were definitely associated with either of these potential hosts was difficult because the burrows are highly networked (Davie, 1992). Sakai (2000) also collected *A. toriumii* from tidal flat where a lot of *Upogebia yokoyai* are distributed but was not able to determine where the crabs lived. Ghani and Tirmizi (1991) reported from the northern Arabian Sea that two female individuals of *Acmaeopleura balssi* were collected attached to the bases of the thoracic appendages of *Upogebia quddusiae*, though the identity of these specimens was doubted by Davie (1992) because the male sub-orbital crest, a diagnostic feature of *Acmaeopleura* crabs, was not mentioned.

In the course of my studies on the symbiotic animals with thalassinidean shrimps, two species of *Acmaeopleura* were collected from burrows of *Upogebia major* (De Haan). One was *Acmaeopleura toriumii* Takeda, and the other was an undescribed species, which was distinctly different from other species of this genus in several morphological characters of carapace and male suborbital crest (Itani, in preparation). I refer to this species as *Acmaeopleura* sp. in this paper. I will describe two different styles of symbioses adopted by the two *Acmaeopleura* crabs and will discuss inhabitable space created by *Upogebia* shrimps. I will use the term "symbiotic" literally in the sense of "living together" in this paper following Ross (1983).

### Materials and Methods

#### *Field collection*

The two species of *Acmaeopleura* crabs were collected in a tidal mud flat in an estuary at Aiofutashima, Ogouri, Yamaguchi Prefecture, Japan (33°59'N, 131°24'E), located in the northwestern part of the Seto Inland Sea. Sampling was conducted from September 1994 to November 1995, roughly bimonthly. During ebb tide, substrate mud with abundant burrows of *Upogebia* was scooped up with a shovel to a depth of 50 cm and sieved. Sometimes the organisms in *Upogebia* burrows were sucked up with a suction pump similar to that described by Manning (1975).

#### *Observation in aquaria*

In the laboratory, the collected crabs and *Upogebia major* were kept in flow-through seawater tanks with mud and gravel at the bottom. At the beginning, each tank contained one or two individuals of *Acmaeopleura* sp., about 15 of *Acmaeopleura toriumii*, and about five of *U. major*. Pieces of clam or fish were added to the tanks about every three days as possible foods for the crabs and enrichment of mud. Some individuals of *U. major* were kept in U-shaped, transparent, vinyl tubes only slightly larger in diameter than that of the shrimp. Thus, the behavior of the crabs and the host shrimps could be observed under burrow-like conditions. Observations on the behavior of the crabs to the shrimp were made for at least four months; most individuals of both crab species outlived observation period, but most shrimp died within two months. Shrimps were added to the tank after the next sampling had conducted. A total of 10 individuals of *Acmaeopleura* sp. and more than 50 of *Acmaeopleura toriumii* were observed. Oviparous females of the host were not used in this study because they rapidly died in aquaria.

To observe the way *Acmaeopleura* sp. clings to the host, 10 individuals of *Acmaeopleura* sp. isolated from their hosts and 20 individuals of *Acmaeopleura toriumii* were put individually into vinyl tubes containing *Upogebia major*. Behavior of the crab and the shrimp was observed until the crab either succeeded to cling to the shrimp or failed to cling it and was cast out of the vinyl tube. Observations were made 10 times for each crab.

#### *Field experiment and distribution of Acmaeopleura toriumii*

In order to clarify that *Acmaeopleura toriumii* is not a burrowing species, a field experiment was conducted at the mud flat at Yamaguchi Prefecture in August 1997. Twenty-three *A. toriumii* were collected with a shovel and placed on the nearby sediment surface at low tide. Crabs were

kept individually in polyvinylchloride pipes (6 cm in diameter and 10 cm long). Each pipe was placed on the mud with one end to the mud surface with a crab and the other end covered with 1 mm-meshed net. After 24 hours, presence or absence of the burrows and the crabs were checked.

So as to determine that *Acmaeopleura toriumii* is not surface dweller in mud sediment, field samplings were conducted at the mud flat at Yamaguchi Prefecture in August 1997. Surface sediment (20×20 cm) with abundant *Upogebia* burrows was scooped with a shovel to a depth of 3 cm, sieved and checked for the presence or absence of *A. toriumii*. The sediment samplings were conducted 100 times at both low and high tide.

## Results

### Field collection

More than 100 specimens of *Acmaeopleura toriumii* (carapace widths, 2.0–8.1 mm) were collected from September 1994 to November 1995. In many cases burrows collapsed just after digging the sediment and I was not able to determine whether *A. toriumii* was associated with *Upogebia* burrows. In some cases, however, individuals of *A. toriumii* were observed associated with the *Upogebia* burrows (Fig. 1). Some individuals of *A. toriumii* were also sucked directly out of the burrows by a suction pump. In March 1995, four individuals of *A. toriumii* were found among egg masses of an ovigerous female of *U. major*. As for *Acmaeopleura* sp., 14 specimens (carapace width, 2.2–8.2 mm) were collected. Eight individuals of *Acmaeopleura* sp. were found clinging to the abdomen of *U. major* and six were found from the sieved sediment.

### Observation in aquaria

Specimens of *Acmaeopleura toriumii* stayed under pebbles or on the mud, and did not make burrows. The crab ate pieces of clam and fish, and also ate dead shrimp and exuvia of the shrimp. The crab sometimes used its chelipeds to scoop mud toward its mouth and its third maxillipeds for filter feeding. Animals of this species ( $n > 50$ ) never clung to *Upogebia major*.

Every specimen of *Acmaeopleura* sp. ( $n = 10$ ) that was placed in an aquarium with *Upogebia major* found a shrimp and clung to it; even the smallest specimen (carapace width = 2.2 mm) behaved in this way. The crab usually clung to the ventral side of the first and second abdominal segments of the host shrimp, using the dactyls of the second, third, and sometimes fourth pereopods as hooks; its head was facing toward the telson of the host (Fig. 2). In a few cases, the crab clung to the ventral side of the sixth abdominal segment. The host shrimp sometimes scraped the crab with its fifth pereopod, but the crab was never observed to leave. The crab sometimes migrated to another host individual, though whether the crab was forced to leave by the host or the crab left for another reason was not determined.

*Acmaeopleura* sp. used its chelipeds to pick some material from the host body and carried it to the mouth. A few days after a crab had attached to a host, a small wound appeared on the abdomen of the host shrimp. The wound expanded daily, and in some cases the first pleopods of female hosts were lost (Fig. 3). Because the wounds reached to the epidermis of the shrimp ( $n = 14$ ), I concluded that the crabs feed on the host tissue, as well as on surface debris. Of the 333 specimens of *Upogebia major* collected from September 1994 to May 1995, 43 specimens (12.9%) had abdominal wounds like those produced by crabs in the laboratory.

When put into a vinyl tube containing *U. major*, every individual of *Acmaeopleura toriumii* ( $n = 20$ , 200 observations) was soon detected by the shrimp, picked up, and cast out of the vinyl burrow. On the contrary, *Acmaeopleura* sp. succeeded to cling to the shrimp in more than half the observations ( $n = 10$ , 100 observations). In 27 of 100 observations, *Acmaeopleura* sp. put into the tubes were found by *U. major*, picked up, and cast out of the vinyl burrow. In the other 73 cases, a crab stole up toward the host from behind and kept still under the abdomen and tried to cling to the host when the host moved. In 57 out of 73 cases, a crab scrambled up the second pleopods

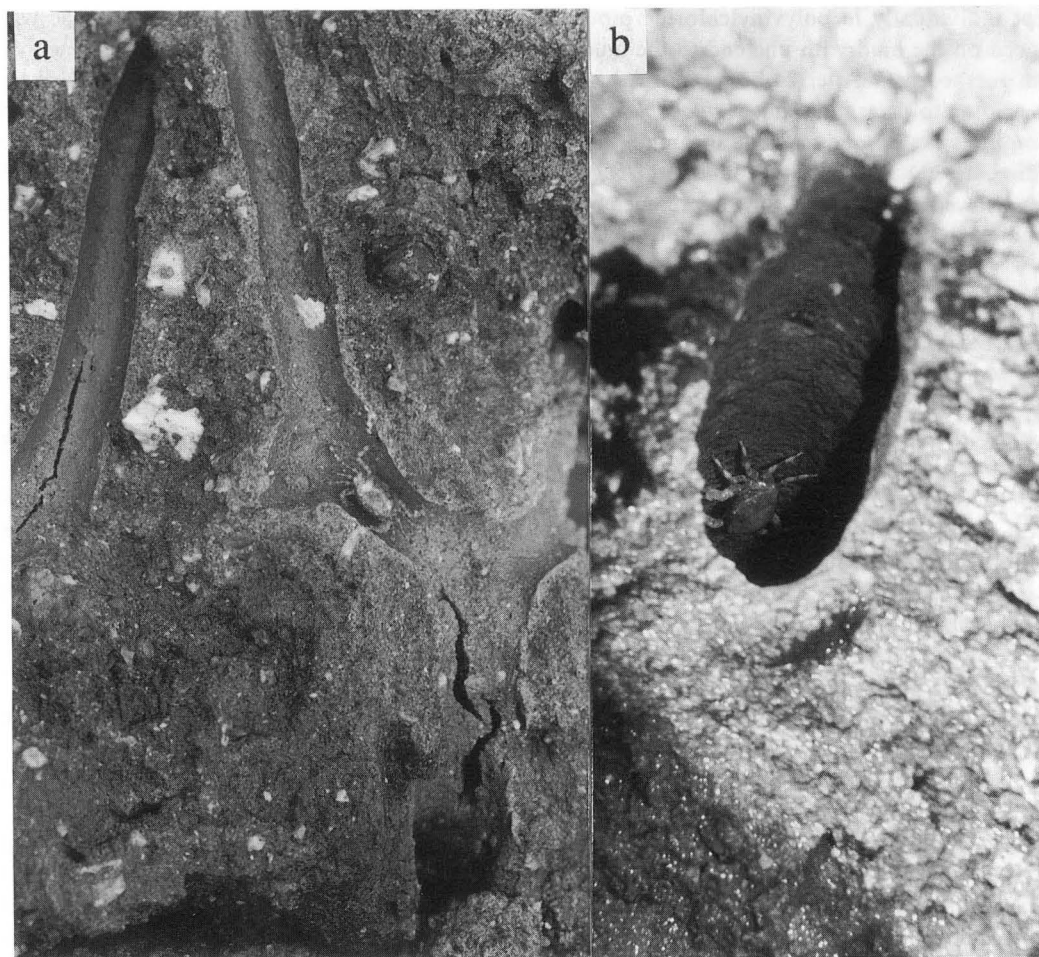


Fig. 1. *Acmaeopleura toriumii* in the burrows of *Upogebia major*. Substrate mud was dug carefully with a shovel and burrows of *Upogebia* were exposed. a: A female (carapace length=6.2 mm). b: Another female (carapace length=7.5 mm).

or the fifth pereopods of the host and were able to cling to the first and second abdominal segments. In other 9 cases a crab climbed the telson and clung to the sixth abdominal segment, and in the other 7 cases a crab failed to climb the host and was cast out of the burrow by the host.

#### Field experiment and distribution of *Acmaeopleura toriumii*

Twenty-four hours after 23 individuals of *Acmaeopleura toriumii* were placed on the mud surface, 19 were found buried in the mud surface, without making their own burrows. Fourteen of the 19 crabs were found at 0~1 cm depth; four at 1~2 cm; one at 2~3 cm. Four individuals were not found in the sediment. But in all the four cases, burrow entrances of *Upogebia major* were found on the sediment covered with polyvinylchloride pipes and missing crabs would have entered to the *Upogebia* burrows. As a result of surface sediment samplings, no individual of *Acmaeopleura toriumii* was found at low tide or high tide.

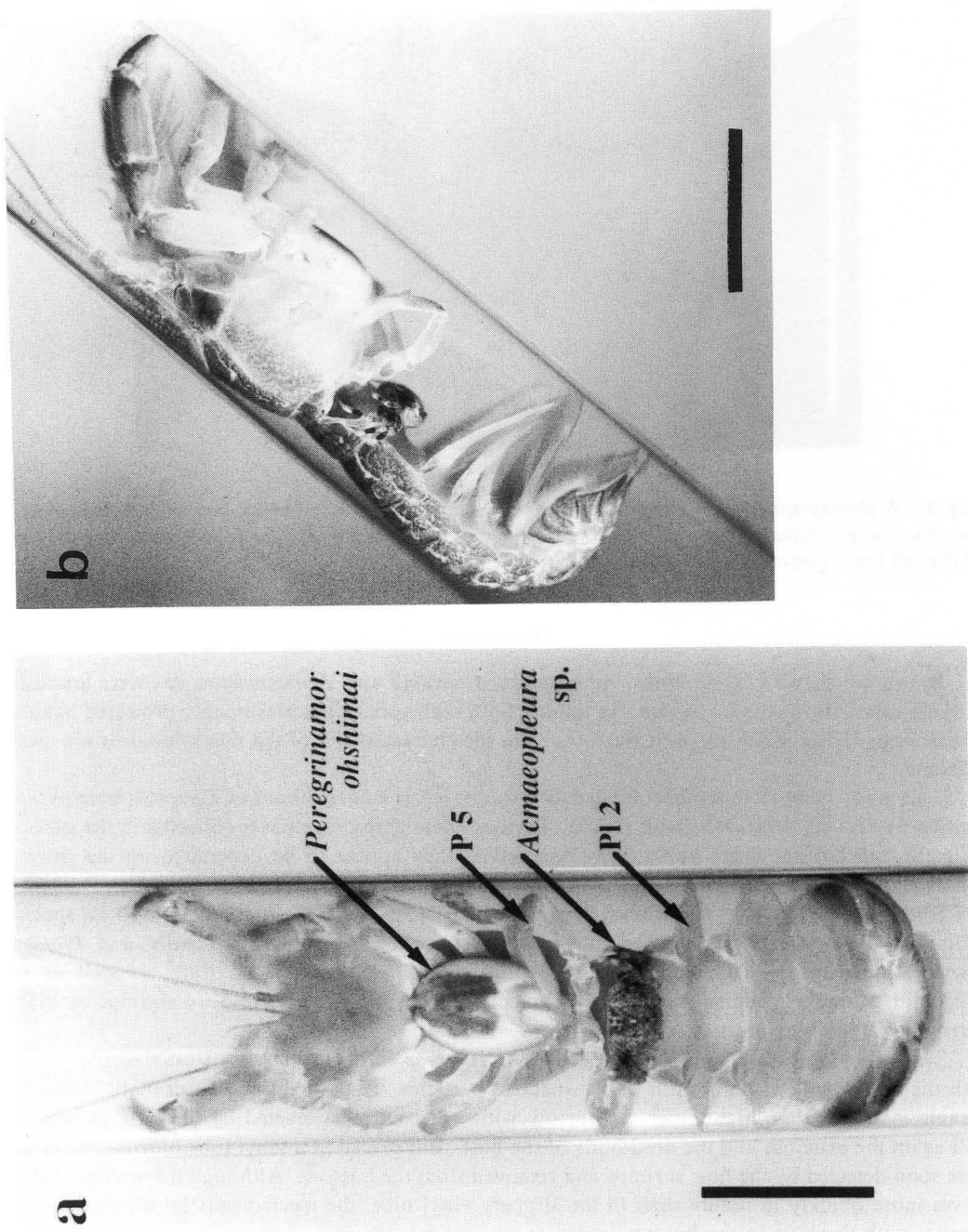


Fig. 2. A living male specimen of *Acmaeopleura* sp. clinging to the host *Upogebia major* (put in a glass tube for photographs). The host harbors the symbiotic bivalve *Peregrinamor ohshimai* as well. a: Dorsal view of the crab. b: Side view of the crab. P5; the fifth pereiopod of *U. major*, Pl2; the second pleopod of *U. major*. Scale bar=10 mm.

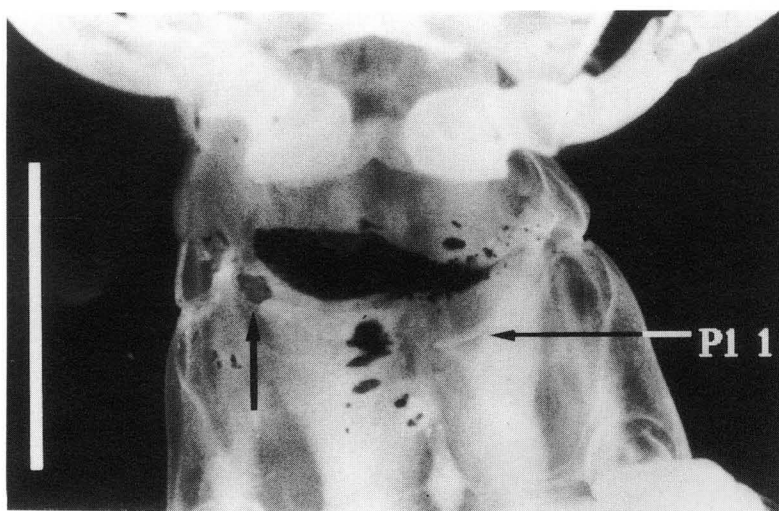


Fig. 3. A photograph of the wounded abdomen of a female host shrimp twenty days after *Acmaeopleura* sp. has clung in aquaria. See the right first pleopod of the shrimp lost by the crab feeding (arrow). P1 1; the left first pleopod. Scale bar = 10 mm.

### Discussion

In this study two grapsid crabs, *Acmaeopleura toriumii* and *Acmaeopleura* sp., were found to be symbiotic with *Upogebia major*. In nature, both crab species are presumably protected against predators by living inside the host burrows. But the characteristics of the two symbioses are quite different.

This study demonstrated that *Acmaeopleura toriumii* is a co-habitant of *Upogebia* burrows, as implied by Davie (1992) and Sakai (2000). Because these crabs were never collected in the surface sediment and did not make burrows by themselves, they appear to be dependent on the shrimp burrows. *Acmaeopleura toriumii* had previously been found in a few localities other than Onagawa Bay (the type locality in the northeast Japan); the rarity of this crab might be due to its special habitat. MacGinitie (1935) showed that pinnotherid crabs, *Scleroplax granulata* and *Pinnixa schmitti*, were burrow commensals of an echiuroid and a callianassid shrimp, as well as an upogebiid shrimp. Whether *A. toriumii* is associated with other thalassinidean shrimps or other burrowing animals is under study.

The behavioral observations suggested that *Acmaeopleura toriumii* is an omnivorous feeder with the ability to feed on detritus and suspended particles. In nature, the crab probably feeds on organic materials that might enter the burrow with water currents created by the host shrimp, as well as on the exuvium and the dead body of the host. But placed in a vinyl tube burrow, the crabs were soon detected by the host shrimps and removed from the burrow. Although the crab probably moves more quickly in nature than in the slippery vinyl tube, the mechanisms by which the crab coexists with the host in the burrow in nature are not known.

The life of *Acmaeopleura* sp. seems to depend on the association with *Upogebia*. The crabs were collected clinging to the abdomen of the host *Upogebia major* and they always clung to the host in aquaria. Crabs that were collected from sediments containing *Upogebia* burrows had probably been detached from the body of their host when the sediments were dug and sieved. Although housekeeping activities of *Upogebia* shrimp are high, *Acmaeopleura* sp. will flee from them clinging directly to the shrimp body. Even if detached from a host, *Acmaeopleura* sp. would



migrate to a nearby burrow and cling to another host individual.

Crabs in aquaria were thought to feed on tissue from the ventral surface of the abdomen of the host. This feeding habit of the crab was not an artifact caused by their maintenance in aquaria, considering about 13% of the collected specimens of *U. major* in the field had an injured abdomen attributable to the crab. But whether the living host tissue is the primary diet was uncertain, because the crab was thought to feed on debris on the host body as well. Feeding experiments and gut content analyses of the two crabs are under study.

The second to fifth pleopods of *Upogebia* shrimps are foliaceous and create the water currents necessary for feeding and ventilation. The first pleopods of the male are absent, and those of the female are simple and small, carrying eggs in the breeding season. There is, in both sexes, a large space between the fifth pereopods and the second pleopods. Usually the crab stays in that space (Fig. 2), a convenient location for clinging to its host: First, the crab is not pressed to the burrow wall when the host shrimp moves. Second, the crab is not disturbed by the beating pleopods of the host. Third, the host cannot use the cleaning legs (i.e. the fifth pereopods) effectively to scrape off the crab, which is lying just behind the legs. Fourth, the ventral abdominal cuticle of the host shrimp is not hard, like the exoskeleton of the cephalothorax and dorsal abdomen, so the crab can cling and feed more easily. Finally, the foliaceous pleopods of the host create an upwelling near the base of the second pleopods that brings fresh water and detrital matter to the crab. Such an upwelling was observed near the bases of the first foliaceous pleopods of a stomatopod, *Oratosquilla oratoria*, to which a galeommatid bivalve *Pseudopythina subsinuata* was attached (Morton, 1972). The space between the fifth pereopods and the second pleopods of *Upogebia pugettensis*, a species in the eastern Pacific, is utilized by the bivalve *Pseudopythina rugifera* and *P. compressa*, and the bopyrid isopods *Phyllodurus abdominalis*, but not by crabs (MacGinitie, 1935).

Ghani and Tirmizi (1991) reported that two females of *Acmaeopleura* crab were collected attached to the bases of thoracic appendages of *Upogebia quaddusiae* in the Arabian Sea. The reported habit of the crab is interesting but rather curious in that the crab does not appear to locate in the space on the first and second abdominal segments which is used by *Acmaeopleura* sp., bopyrid isopods, and bivalves. The ventral side of the cephalothorax of *Upogebia* shrimps is usually free of other animals, except for the highly specialized, symbiotic bivalves *Peregrinamor ohshimai* and *P. gastrochaenans*, that attach there by its byssus and uses the anterior inhalant siphon to pilfer the suspended matter captured by the setal basket of the hosts (Kato and Itani, 1995, 2000). In the absence of special feeding habits, the ventral cephalothorax of shrimps would seem to be inhospitable for crabs. Behavioral and systematic studies of the *Acmaeopleura* crabs in the Arabian Sea are required.

The case of a decapod ectosymbiont on another decapod is extremely rare (Ross, 1983). Common hosts of decapod symbionts are sedentary or less mobile animals, such as cnidarians, molluscs, annelids and echinoderms (Ross, 1983). Exceptional cases are some species of porcellanid crabs which live inside the shells of live gastropods and hermit crabs (Telford and Daxboeck, 1978; Werding, 1983), and the alpheid shrimp *Aretopsis amabilis* which lives in the hermit crab shell and eats the faeces of the host (Bruce, 1969; Kamezaki and Kamezaki, 1986). In both cases, the decapod symbionts will be considered as shell co-habitants and may be comparable to burrow co-habiting shrimps and crabs. Thus, *Acmaeopleura* sp. - *Upogebia* symbiosis would be a novel case of symbiotic relationship in decapod crustaceans in that the crab lives ectoparasitic with a shrimp. *Acmaeopleura* sp. - *Upogebia* symbiosis could be realized by the fact that upogebiid shrimps are rather motionless animals once having established their burrows and the fact that grapsid crabs are quick in their movement. Further investigations must examine morphological and ecological differences between *Acmaeopleura* crabs, and the relative costs/benefits and the specificity of the symbiotic relationships.



### Acknowledgements

I express my gratitude to Y. Shirayama, E. Harada, and M. Kato who read the manuscript and supported all aspects of this study. I especially thank A. Taki, M. Takeda, K. Wada, and P. Davie for providing information about the crabs; S. Nishimura and R. Asai for technical assistance; M. Imafuku, Y. Fukui, and K. Iwasaki for helpful comments on this study; and K. Yoshimura, T. Inoue, and Y. Itani for field assistance. Thanks are also due to A. Mori and T. Uchino who provided valuable literature, and all the other members of the Seto Marine Biological Laboratory and the Laboratory of Animal Ecology of Kyoto University who helped me with the experiments and observations. I would like to thank M. J. Greenberg and anonymous reviewers for constructive comments on the earlier version of the manuscript. This study was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and a JSPS Fellowship for Young Scientists.

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Submitted papers must not have been published nor be under consideration for publishing elsewhere in any languages. The submitted papers will be reviewed at least by two referees, and only those papers evaluated their scientific findings are worth for publication will be accepted. Such papers that is judged not paid sufficient attention to the wildlife conservation and the humane treatment of animals will be rejected.

Manuscript should be sent to the editor in chief (Yoshihisa Shirayama) of the Publications at the following address:

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Authors are requested to read the following instruction carefully, and prepare their manuscript properly. Poor attention to the instruction may cause unnecessary delay of the processing of papers.

Manuscripts should be prepared in English as concisely as possible. Those authors whose native language is not English should ask native English speaker to correct language usage before submitting the manuscript. Original and two copies of the manuscript consisting of text, tables and the illustrations should be provided.

### **Text:**

The text should be printed in double spaced manner on one side of A4 size paper leaving margins of more than 3 cm in width all around. All sheets should be numbered consecutively, and the name of the senior author should be given on the upper right corner.

The first page of the text should include the title of the paper, information of author(s) (name, affiliation, and corresponding address inclusive of e-mail address of all authors and telephone and fax numbers of the representative author), the running title (not more than 50 characters), and key words (not more than 10 items).

The second page should include only the abstract of the paper (less than 300 words), expressing concisely the hypothesis, the approach the author(s) adopted, the results, and the conclusion.

The main body of the text consisting of introduction, materials and methods, results, discussion, acknowledgments, reference and so on, should be prepared from page 3. The papers listed in the reference section should be referred as Tokioka (1960, 1961a) or (Harada, 1995; Harada *et al.*, 1992; Harada and Fuse, 1994).

The reference should list all the papers mentioned in the text alphabetically in the following manner.

### *Referring papers published in journals:*

Shirayama, Y. 1984a. The abundance of deep-sea meiobenthos in the Western Pacific in relation to environmental factors. *Oceanologica Acta*, 7, 113-121.

Shirayama, Y. 1984b. Vertical distribution of meiobenthos in the sediment profile in bathyal, abyssal and hadal deep-sea systems of the Western Pacific. *Oceanologica Acta*, 7, 123-129.

Shirayama, Y. and Horikoshi, M. 1989. Comparison of the benthic size structure between sublittoral,

upper-slope and deep seas of the Western Pacific. *Internationale Revue der gesamten Hydrobiologie*, 74, 1–13.

(It should be noticed that the title of journals should not be abbreviated.)

*Referring papers published in books:*

Shirayama, Y. 1995. Current status of deep-sea biology in relation to the CO<sub>2</sub> disposal. In, Handa, N. and Ohsumi, T. (eds.) *Direct Ocean Disposal of Carbon Dioxide*, Terra Scientific Publishing Company, Tokyo, pp. 253–264.

*Referring a book as a whole:*

Gage, J. D. and Tyler, P. A. 1991. *Deep-Sea Biology*. Cambridge University Press, Cambridge, 504 pp.

**Tables:**

The tables should be provided in the shape ready to reproduce. Each table should be printed on a separate sheet of paper using a font “Times new roman” or related one. The large table that can not be printed in 2 pages is not acceptable.

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